TREHALOSE FERMENTATION IN THE DIFFERENTIATION OF THE PARATYPHOID-ENTERITIDIS GROUP

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The investigations of Bainbridge,[1] Savage,[2] Jordan,[3] Krumwiede and his associates [4] and others have done much to clarify our knowledge of the confusing paratyphoid-enteritidis group, and as a result of this work a number of well-defined types are now recognized. A summary of this group with suggested changes in nomenclature is given by Winslow, Kligler, and Rothberg.[5] Recently, Ten Broeck [6] has called attention to a group of paratyphoids of animal origin, which he found to be culturally identical to B. paratyphosus B, or B. schottmulleri, but which may be differentiated by agglutinin absorption tests. Many of the paratyphoid B bacilli isolated from food poisoning cases quite possibly belong to the same group and Ten Broeck suggests that "if it is found upon further study that these organisms are the same as the Aertrycke bacillus, isolated by DeNobele, the name of Bacillus aertryckei would be appropriate." An apparently similar group was observed by Krumwiede, Valentine and Kohn [7] who by absorption tests separated from B. schottmulleri a number of strains of rodent origin.

Thus, while the various members of the paratyphoid-enteritidis group can be separated one from the other by serologic methods, the distinctions based on cultural or biochemical features are in some instances slight or altogether lacking. For this reason it is believed that any further means of differentiation may be of distinct practical value. While engaged in identifying an organism of this group asso-

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1. Jour. Pathol. and Bact., 1908-09, 13, p. 443. Bainbridge, F. A., and O'Brien, R. A.: Jour. Hygiene, 1911, 11, p. 68.

2 Jour Hygiene, 1912, 12, p. 1.

3 Jour. Infect. Dis., 1917, 20, p. 457; 1918, 22, p. 511; 1920, 26, p. 427. Jordan, E. O., and Victorson, R.: Idem., 1917, 21, p. 554.

4 Krumwiede, C.; Pratt, J. S., and Kohn, L. A.: Jour. Med. Res., 1916-17, 35, 55, p. 357. Krumwiede, C.; Kohn, L. A., and Valentine, E.: Idem., 1918, 38, p. 89.

5 Jour. Bacteriol., 1919, 4, p. 429.

6 Jour. Exper. Med., 1918, 28, p. 759; 1920, 32, p. 19.

7 Jour. Med. Res., 1919, 39, p. 449.

ciated with a case of human food poisoning, it was observed that certain types could be differentiated on the basis of their abilities to ferment trehalose. It is my purpose to present these results.

Trehalose is a disaccharide sugar consisting of 2 molecules of glucose attached in such a way that both aldehyde groups have disappeared.[8] It does not reduce Fehling's solution. Two different samples of trehalose were available for this study. The one was procured from a commercial firm, the other was prepared in the Carbohydrate Laboratory of the Bureau of Chemistry. Results obtained by the use of either sample were checked by similar tests with the other. The 2 samples gave identical results.

The strains of the several types of the paratyphoid-enteritidis group employed in this investigation were for the most part stock cultures received from various laboratories. The writer is especially indebted to Dr. K. F. Meyer and to Dr. Carl Ten Broeck for a number of the para B type cultures of animal origin. The term "animal para B cultures" is used in this paper to designate the group of paratyphoid B cultures of animal origin which may be differentiated from B. schottmulleri by agglutinin absorption tests.[6, 7] This expression is used here for convenience only, and in the absence of definite knowledge that these cultures are similar to the B. aertryckei of de Nobele. It should be noted that cultures identical to B. schottmulleri have been reported as obtained from animal sources.[9] One of these, 135 "B," is included in the present investigation.

In table 1 is shown the deportment of the various members of the paratyphoid-enteritidis group toward trehalose when incorporated in the ordinary culture mediums. For comparison, results are also given for the test substances usually employed in the differentiation of these types. Here it is seen that B. suipestifer, in contrast to the other members of this group, is incapable of attacking trehalose. On continued incubation of the trehalose broth, an apparent reversion to an alkaline reaction was observed in many of the B. schottmulleri cultures. The brilliant red color of the Andrade indicator gradually faded and eventually disappeared. On the basis of what was probably a similar loss of color in old cultures, Krumwiede, Pratt and Kohn[10] devised their glucose

[8] Armstrong, E. Frankland: The Simple Carbohydrates and the Glucosides, Ed. 3, 1919, p. 101.

[9] Spray, R. S.: Jour. Infect. Dis., 1920, 26, p. 340.

[10] Jour. Med. Res., 1917, 35, p. 357.

serum water medium. This suggested to the writer the possibility of a similar means for differentiating the animal para B cultures from B. schottmulleri.

The first procedure tried for the separation of the two groups referred to in the foregoing was the substitution of trehalose for glucose in Krumwiede's glucose serum water medium.[11] After some experimentation it was found that a sharp distinction could be brought out between B. schottmulleri and the animal para B type, provided that certain conditions were fulfilled. The most desirable procedure thus far found has been to add the trehalose to the serum water in sufficient

TABLE 1

ACTION OF MEMBERS OF PARATYPHOID-ENTERITIDIS GROUP TOWARD TREHALOSE

Types	Number of Strains	Fermentation of							
		Xylose		Arabinose		Dulcite		Trehalose	
		+	−	+	−	+	−	+	−
B. paratyphosus (para A)	3	0	3	3	0	3 (slow)	0	3	0
B. schottmulleri (paratyphosus B)	7	7	0	7	0	7	0	7	0
Animal para B type	14	13 1 (3 days)	0	14	0	14	0	10 2 (48 hrs.) 2 (3 days)	0
B. enteritidis	6	6	0	6	0	6	0	6	0
B. suipestifer	8	8	0	0	8	1 (rapid) 2 (slow)	5	0	8

For these tests meat extract peptone broth, P_H 7.0–7.2, to which was added 1% of Andrade indicator was used. The sugars and the alcohol, dulcite, were sterilized separately in 10% solution in distilled water and added aseptically to the broth tubes in sufficient amounts to give a concentration of 0.5%. The tests were observed for 2 weeks in order to include any delayed fermentation. All fermentation results recorded as positive occurred within 24 hours unless otherwise designated.

quantities to give a concentration of 0.5% and to tube the resulting trehalose serum water medium in small quantities to insure a shallow layer of medium, preferably not more than 1.5 cm. in height. After 3 to 4 days' incubation at 37 C., the coagulum produced by the animal para B cultures presents a deep pink or red color, while that of the B. schottmulleri cultures is colorless or a light pink tint. This distinction is not noticeable after 24 hours, but is gradually brought out on further incubation. It has been the writer's experience that the longer the period

[11] For the convenience of the reader, the composition of this medium is repeated here: one part sterile horse serum and 4 parts sterile distilled water to which are added 1% Andrade indicator and sufficient amounts of a sterile glucose solution to give a final concentration of 0.1%. The medium may be prepared from sterile materials and tubed aseptically or prepared from nonsterile materials and given a 3-day intermittent heating in the Arnold sterilizer.

of incubation, up to one week, the greater is the contrast between the two types. After that time no additional changes have been observed. Most of the animal para B cultures have been found to be somewhat slower in coagulating the serum than are the B. schottmulleri strains. In the case of a few of the animal paratyphoid cultures which required 48 to 72 hours for a noticeable fermentation of trehalose (table 1), the typical appearance in the trehalose serum medium has been delayed for

TABLE 2

SHOWING CORRELATION OF BIOCHEMICAL AND SEROLOGIC DIFFERENTIATION OF THE "ANIMAL PARA B" CULTURES AND B. SCHOTTMULLERI

	Agglutinin Absorption Tests				Fermentation of Trehalose: Serum Water, 1% Andrade Indicator, 0.5% Trehalose. 4 Days at 37 C.
	B. schottmulleri Serum "Rowland" Absorbed by		Calf Typhus 1 Serum Absorbed by		
	Nothing (Control)	Calf Typhus 1	Nothing (Control)	B. schott-mulleri (Rowland)	
B. schottmulleri					
Am. Mus.	6,400	1,600	800	200—	Faint pink
Cool's Army	6,400	1,600	3,200	200—	White
Roanoke	6,400	3,200	1,600	200—	Faint pink
Rowland	6,400	1,600	1,600	200—	White
Jordan 210	6,400	3,200	1,600	200—	Faint pink
F	6,400	1,600	1,600	200—	Faint pink
Perdue, 135 B	6,400	1,600	3,200	400	Faint pink
Animal para-B					
Calf-typhus 1	3,200	200—	6,400	3,200	Red
Swine-typhus 1	3,200	200—	6,400	3,200	Red
Mouse-typhus 1	3,200	200	6,400	3,200	Red
Swine-typhoid 2-1	3,200	200	6,400	1,600	Red
Swine-typhoid 2-2	1,600	200	6,400	3,200	Deep pink
Pigeon 6	3,200	400	6,400	1,600	Red#
Canary 12	6,400	400	6,400	1,600	Deep pink
Psittacosis, Past. Inst.	3,200	200	6,400	800	Deep pink#
Guinea-pig 10	3,200	200	6,400	3,200	Deep pink
Guinea-pig 479	3,200	200	3,200	1,600	Red
Guinea-pig 480	3,200	200	6,400	1,600	Red+
Rabbit 1371	3,200	200	6,400	3,200	Deep pink
Rabbit 45	3,200	200	3,200	1,600	Deep pink+
Anatum (Rettger)	1,600	200	6,400	3,200	Red

Agglutination tests: Immune rabbit serums used. Figures represent the highest dilution at which definite clumping was observed. 200— = no clumping at 1:200. Dilutions lower than 1:200 were not made.

Trehalose serum water: Coagulation of serum in all tubes with the production of the color indicated.

#, + = slow fermenters (table 1); typical appearance delayed 3 days = (#) or 2 days = (+).

a corresponding interval. This deep pink or red color of the animal para B cultures is first apparent at the surface of the coagulum, so that if a deep column of medium is used the typical coloration may appear only in the upper part of the coagulum with the result that much of the sharpness of the distinction is lost. The exact percentage of trehalose necessary to bring about this reaction has not been determined. When 0.1% trehalose was used, all the cultures remained color-

less or a light pink, and the distinction between the 2 types was then not manifest. One series of tests in which a concentration of 0.3% was employed gave a fairly well-defined distinction. In the greater part of this work 0.5% has been used.

Several repeated trials of this method with different lots of horse serum have given consistent results, and in every case the distinction based on this reaction in the trehalose serum water medium has correlated with the grouping by agglutinin absorption (table 2). Further, it is worthy of note that in this medium all the strains of B. enteritidis presented an appearance identical to that of the "animal para B" cultures and in contrast to B. schottmulleri. B. suipestifer, which is unable to attack trehalose, produced no change in the medium. The few B. paratyphosus cultures used in this investigation have coagulated the medium with the immediate production of either a deep pink or red color, i. e., no reduction of the Andrade indicator.

The hydrogen-ion concentration attained in a medium consisting of 0.5% peptone, 0.5% dipotassium hydrogen phosphate, and 0.5% trehalose has been found to be of some value in separating the animal para B cultures from B. schottmulleri, although it has not given as reliable and consistent a demarcation as has the trehalose serum water. Different peptones, Witte and Difco, have yielded different results. Also, the 4 cultures previously noted as slow fermenters of trehalose are exceptions and do not permit a uniform separation of these 2 types. When Witte peptone was used in the medium referred to in the foregoing the majority of the animal para B cultures attained a hydrogen-ion concentration of 5.0 to 5.2 after 48 hours at 37 C., while that of the B. schottmulleri strains varied from 5.6 to 5.8. On the addition of 4 or 5 drops of a 0.02% solution of methyl red to tubes containing 4-5 c c of a 48-hour culture, the animal para B cultures, with the exception of the slow fermenters, exhibited a pink color, in contrast to the light orange of the B. schottmulleri cultures. When Witte peptone was replaced by Difco, different results were secured with different lots of the latter brand of peptone. With one lot the results were quite similar to those obtained with Witte peptone. In others (a different bottle of the Difco peptone) four days' incubation were required before a distinct separation could be brought out between the two types. In this case, the B. schottmulleri cultures were yellow in contrast to the pink of the majority of the animal para B types. All of the strains of B. enteritidis throughout the numerous trials of this medium have attained

a hydrogen-ion concentration similar to that of the "animal para B type" and in contrast to B. schottmulleri. Because of the several discrepancies mentioned, this method is not recommended as one for classification of these types, although the results, especially when considered with those obtained by the use of the trehalose serum water medium, are suggestive.

SUMMARY

In a study of the availability of trehalose for the various members of the paratyphoid-enteritidis group, it was found that B. suipestifer is unable to attack this disaccharide, whereas B. paratyphosus, B. schottmulleri, the animal para B sub-group, and B. enteritidis ferment trehalose with the production of acid and gas.

Furthermore, it is possible to differentiate by cultural methods the B. schottmulleri strains from the closely allied animal para B group, hitherto separable only by serologic means. This has been accomplished by employing small amounts of a serum water medium containing 0.5% trehalose and 1% Andrade indicator. In this medium the animal para B strains produce a red coagulum after 3 to 4 days' incubation, while the B. schottmulleri cultures present a light pink or colorless coagulum. This separation has been found to parallel the differentiation of these 2 groups by agglutinin absorption tests. B. enteritidis is similar in its reaction to the animal para B cultures and in contrast to B. schottmulleri.